CYTOLOGY

بسم الله الرحمن الرحيم

MID – Lecture #8

The Cytoskeleton and Cell movement

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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Lecture 6: the cytoskeleton and cell movement (Microtubules and intermediate filaments)

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Second year, Second semester, 2024-2025

Overview

Empty inside

- Microtubules are rigid hollow rods.
- They are dynamic structures that undergo continual assembly and disassembly within the cell.
- Functions:
  - Cell shape
  - Intracellular transport of organelles
  - Separation of chromosomes during mitosis
  - Cell movements (some forms of cell locomotion)

Important in the transport of molecules particularly organelles specifically vesicles

Lysosomes, endosomes, secretory vesicles and so on ..

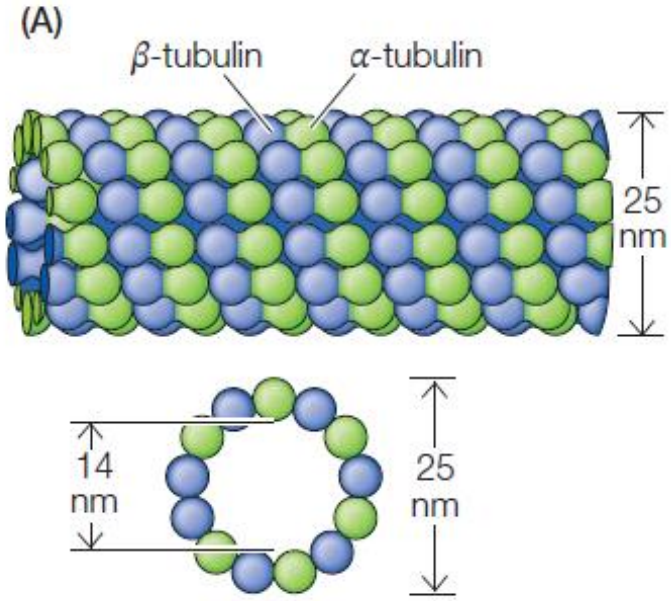
During cell division

Cell movement depends on actin cytoskeleton, but microtubules are essential for some of them .

Structure of microtubules

- Microtubules are composed of a dimer of two globular proteins, α-tubulin and β-tubulin.
  - γ-tubulin is specifically localized to the centrosome and it initiates microtubule assembly for chromosomal separation.
- The tubulin dimers polymerize to form protofilaments (a hollow core) of head-to-tail arrays of the tubulin dimers.
- Both α- and β-tubulin bind GTP.

*GTP that is bound to (β-tubulin) is the one which is responsible for the function, stability and structure of the microtubule.



Formation happens by the binding between the dimers

α-tubulin & β-tubulin

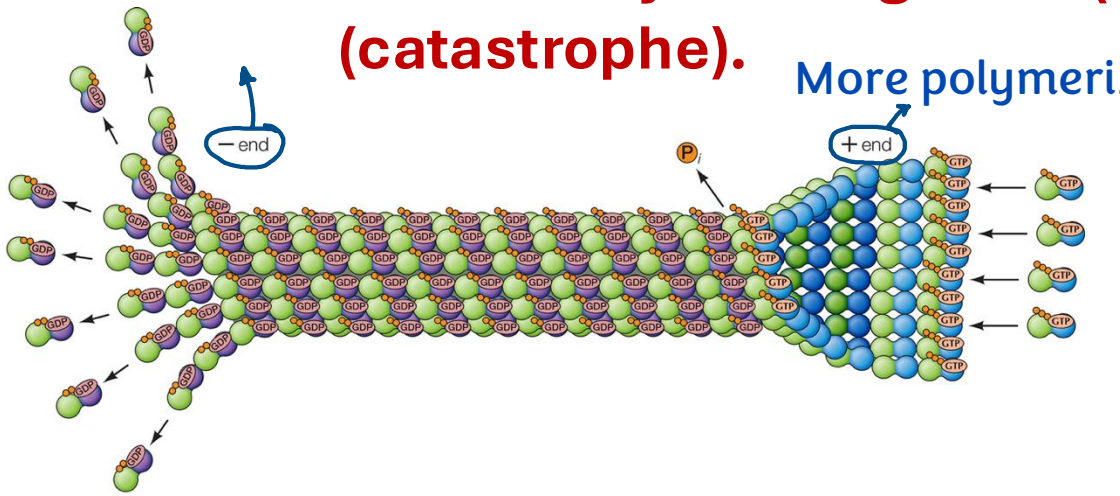
So we have alternating binding between tubulin dimers

Treadmilling and dynamic instability

- Microtubules are polar structures with a fast-growing plus end and a slow-growing minus end.
  - Polarity determines the direction of movement along microtubules.
- Microtubules undergo assembly and disassembly (treadmilling) where tubulin molecules are lost from the minus end and replaced by the addition of tubulin molecules bound to GTP to the plus end.

Dynamic instability: The alternation of microtubules between cycles of growth (rescue) and shrinkage (catastrophe).

More depolymerization



More polymerization

*We will focus on GTP bound to BETA tubulin.

Explanation is in the next slide

Further explanation for the previous idea!

- When the binding happens the GTP that is bound to (BETA-tubulin) is hydrolyzed into GDP, which makes the dimer (BETA) unstable so it is gonna dissociate as shown in the previous figure.
- Microtubule alternates between polymerization (for both α & β tubulins) and depolymerization (for both dimers) → **(dynamic instability)**
- Polymerization and depolymerization happen at both ends, but after hydrolysis of GTP that is bound to (BETA-tubulin) into GDP depolymerization will dominate at one end VS the other end.. just like the actin.. so we have (- end / + end)
- when we have more stability of GTP bound to (BETA-tubulin) → so we have a growth.
- when we have more hydrolysis of GTP bound to (BETA-tubulin) → so we have shrinkage.
- It is a balance between polarization and depolarization

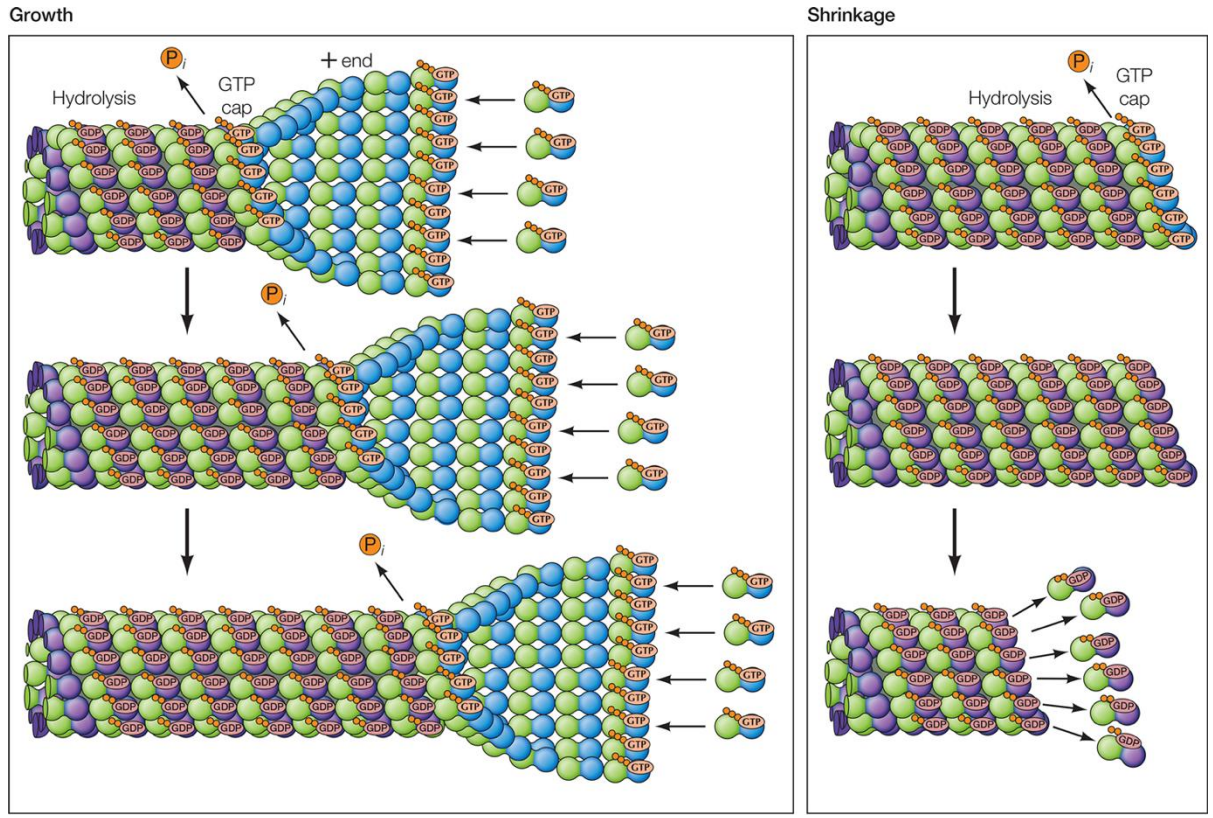
- If binding is faster, the microtubule will get larger & if dissociation is faster, there will be shrinkage
- When I have too much shrinkage it is called catastrophe (كارثة), like domino effect!
- Treadmilling is used to express the continuous alternating process.

The reason behind dynamic instability

- Dynamic instability results from GTP hydrolysis of β-tubulin during polymerization, which reduces its binding affinity for neighboring molecules.
- Growth of microtubules continues as long as new GTP-bound tubulin molecules are added more rapidly than GTP hydrolysis.
- Faster GTP hydrolysis than the addition of new subunits leads to the disassembly and shrinkage of microtubules.

Growth

Shrinkage



The main driver is GTP hydrolysis for growth and shrinkage,
So hydrolysis affects binding affinity between BETA dimers together
→ So dissociation happens.

A brief introduction to drugs.. Easy!

- Zaman, drugs were discovered by extracting some chemicals randomly from different natural environments and put it inside cells and see the effect.
- Now scientists are having the ability to understand the mechanisms of even a small molecule that stimulates certain function –> so they prepare a specific inhibitor for it to stop an inflammation for example.. (targeted therapy)
- They are able to Predict a protein structure, and do modeling for the protein and for the drug to make some trials.
- So drug discoveries became faster and more specific ,and side effects decreased.

They found that microtubules are very important for cell life and division so they discovered some natural drugs from plants.

Drugs

- Colchicine and colcemid bind tubulins, inhibit polymerization, and block mitosis.
- Vinblastine and vincristine bind to tubulin and prevent their polymerization to form microtubules.

Is it Bad? Yes

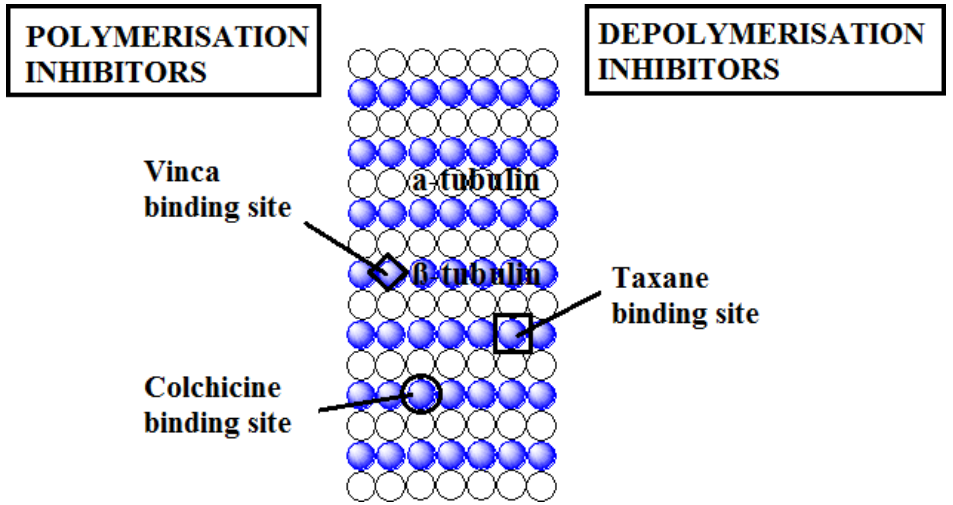
- Taxol stabilizes microtubules and blocks cell division.

inhibit the polymerization by binding to the dimers.

Taxol stabilizes the polymerization (Stuck)

Colchicine & colcemid are experimental drugs (we use them in labs more than on patients) although colchicine is used for immune diseases such as rheumatoid arthritis.

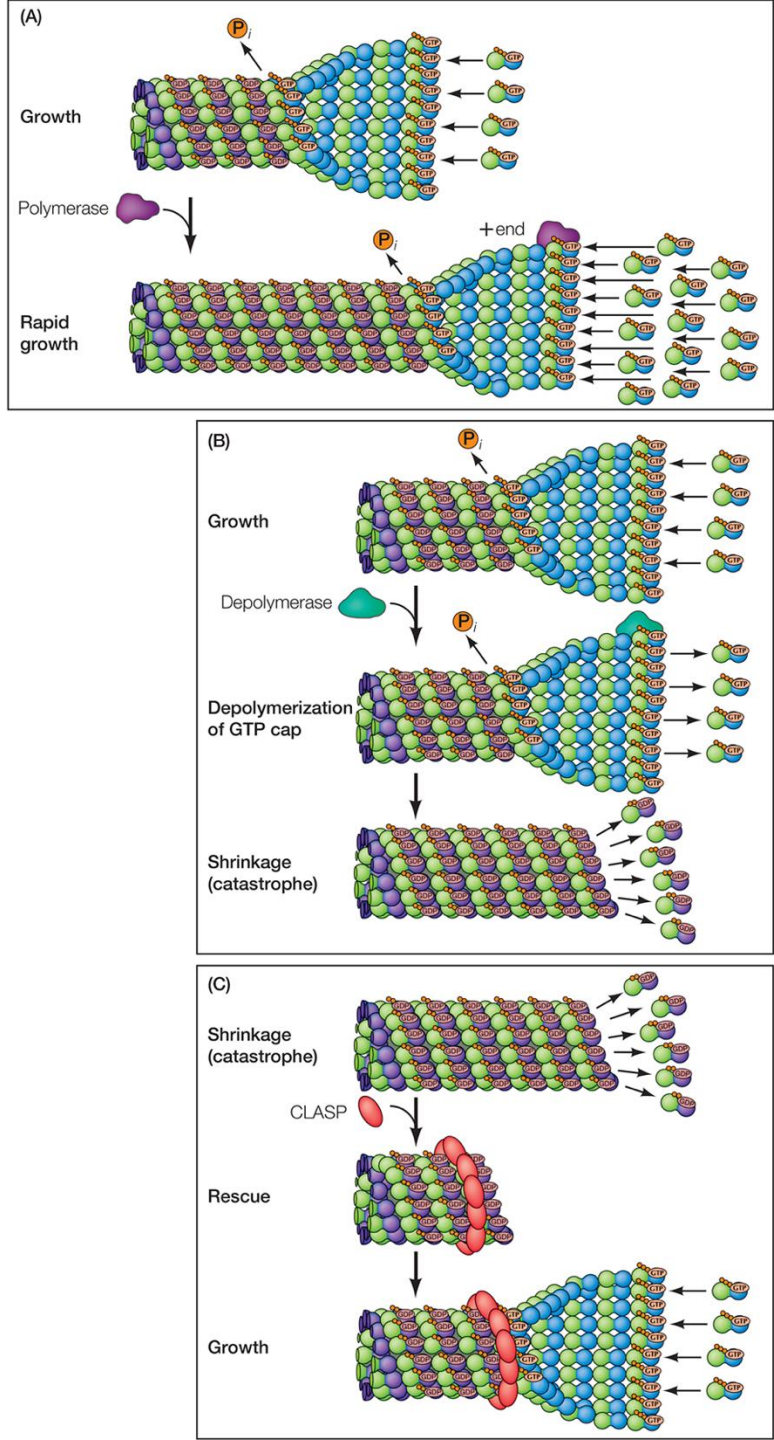
Vinblastine & vincristine are used in cancer chemotherapy with huge side effects.



Regulatory proteins

- Microtubule-associated proteins (MAPs) regulate the dynamic behavior(GTP hydrolysis, growth, and shrinkage)of microtubules by
  1. Regulating:
     A. growth or polymerization (by polymerases) or
     B. shrinkage or depolymerization (by depolymerases) at the plus ends of microtubules.

Regulation is driven by the activation or inhibition of enzymes
(✓ polymerases/ × depolymerases-> polymerization
× polymerases/ ✓ depolymerases-> depolymerization)

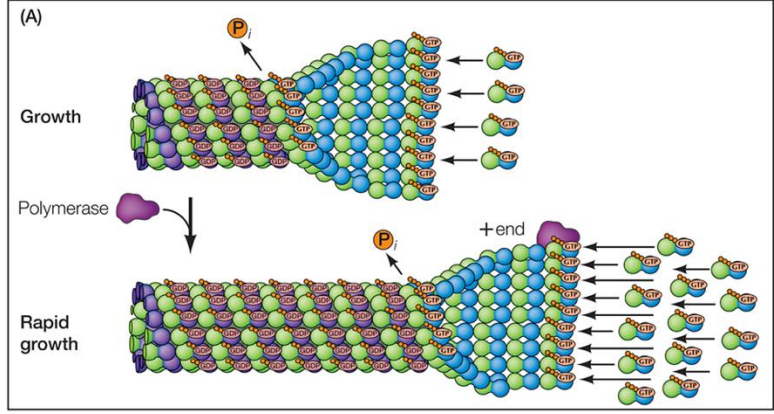


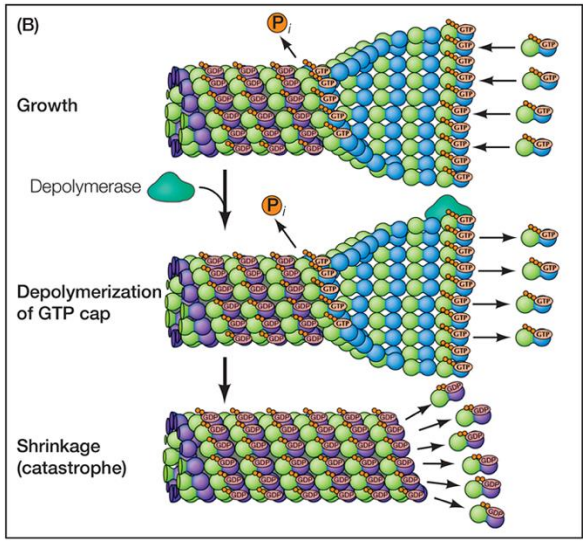
Regulatory proteins

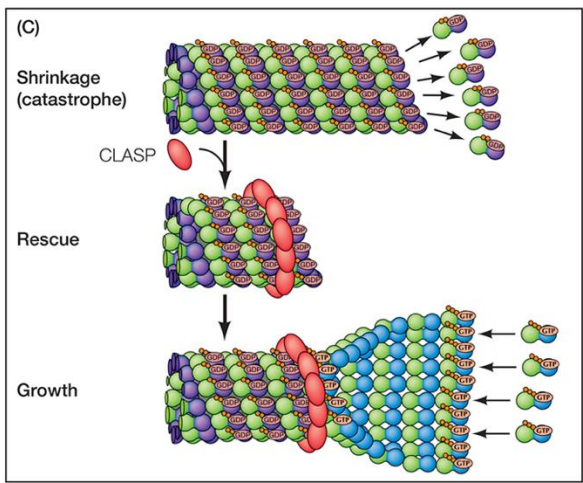
- Microtubule-associated proteins (MAPs) regulate the dynamic behavior of microtubules by

2. Suppressing microtubule catastrophe and promoting rescue:
    - CLASP proteins rescue microtubules from catastrophe.

*Catastrophe: quick and rapid shrinkage by depolymerization.







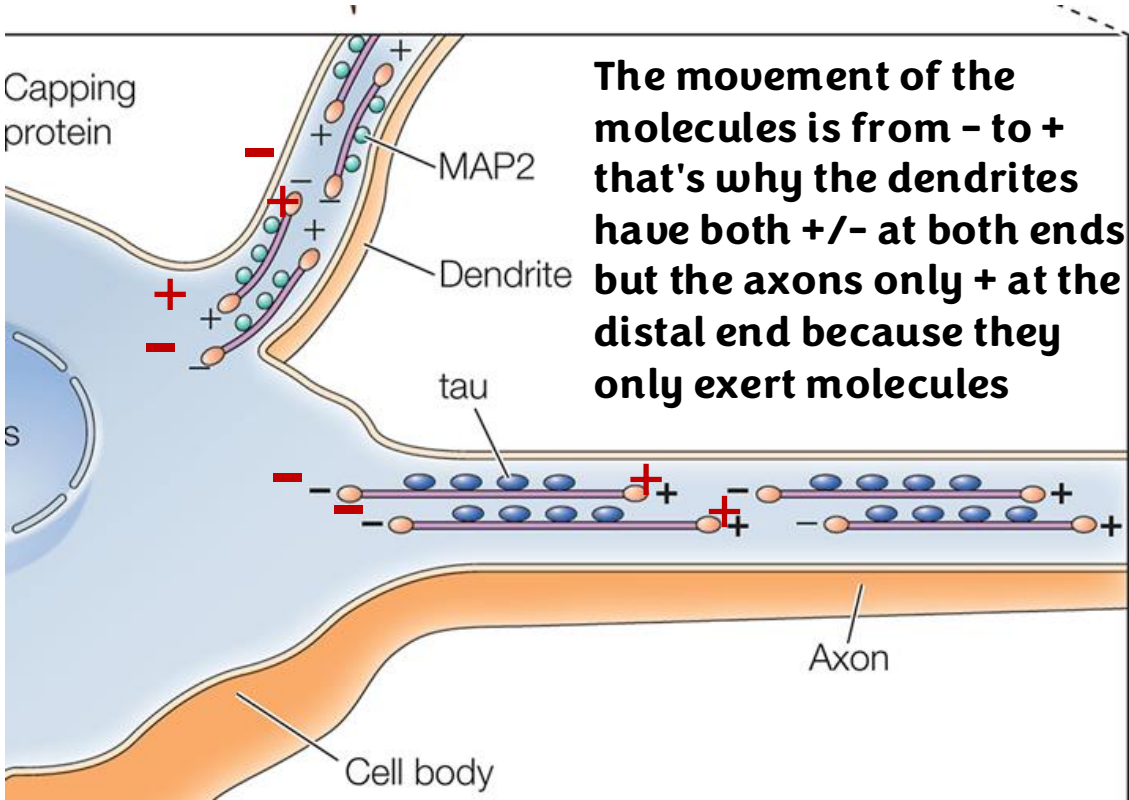
Organization of microtubules within cells Example: neuron

- Neurons have two types of processes(extensions)that extend from the cell body:
  - Dendrites: short; receive stimuli(signal) from other nerve cells(neighboring cells)
  - Axon: long; carries impulses(vesicles filled with neurotransmitters or the enzymes that synthesize NT) from the cell body to other cells the transmission of the vesicles is facilitated by microtubules

Organization of microtubules within cells Example: neuron

- The plus and minus ends of microtubules in nerve cells terminate in the cytoplasm.
- In dendrites, microtubules are oriented in both directions. the plus end at the cell body and the minus at the dendritic end or vice versa.
- In axons, microtubules are oriented with their plus ends pointing toward the tip of the axon.
  - Axons contain tau protein(it's a regulatory protein-MAP), which is the main component of one of the characteristic lesions found in the brains of Alzheimer's patients(because this protein aggregates inside the nerve cell leading to the cell's death causing Alzheimer's disease and its associated symptoms; dementia

Do you recall what other protein misfolding is associated with Alzheimer's? Amyloid! This protein abnormally cleaves from the cell surface and leaves it and it aggregates (clusters/ amyloid plaques) which causes cell damage



The movement of the molecules is from - to + that's why the dendrites have both +/- at both ends but the axons only + at the distal end because they only exert molecules

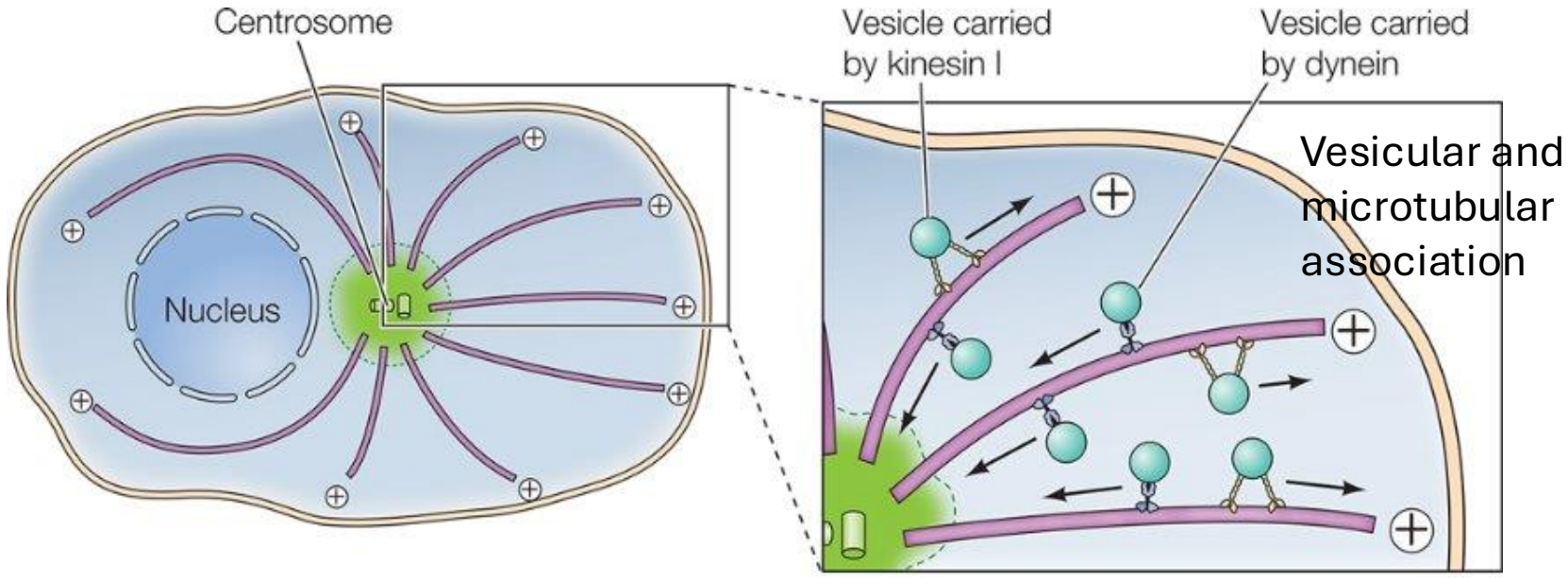
Microtubules-motor proteins(the transporters of molecules inside the cells)

e.g., kinesin and dynein

- Microtubules-motor proteins use ATP to move along microtubules in opposite directions.
- Remember the axonal transport(anterograde and retrograde.)
- Kinesin moves toward the plus end. (click to see the movement) it carries the molecules/ vesicles from the cell body to the axonal end(+ end) till it leaves the cell.
  - Dynein moves toward the minus end. So in the dendrites it moves/ carries molecules from the dendritic end towards the cell body

Part of the molecules bind microtubules and the other part binds to the vesicle or organelle.

Disclaimer : Exceptions

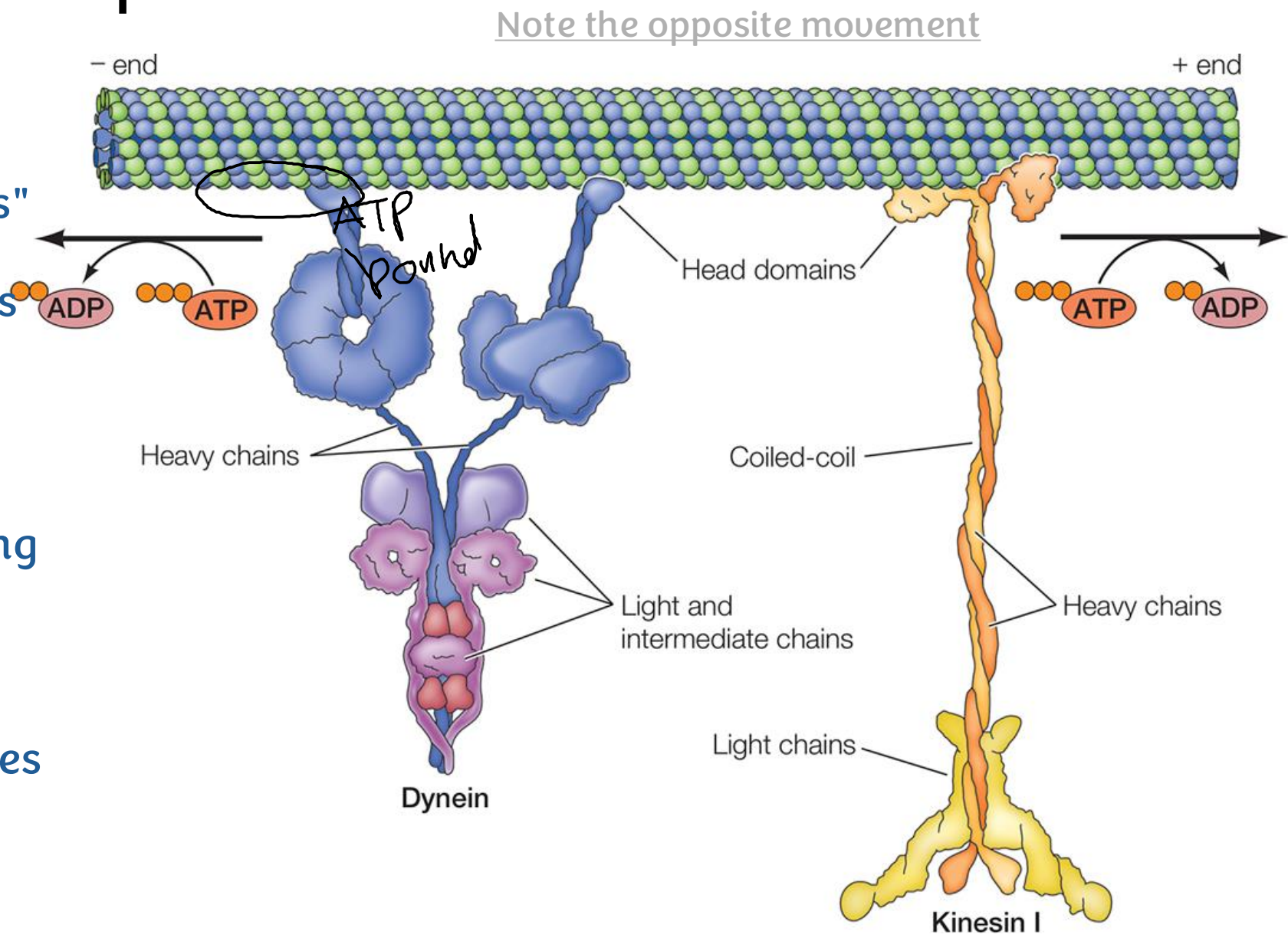


Microtubules-motor proteins

Let's take a closer look:

They have a complex structure that uses ATP to move their" legs" that associate with the microtubules, their movement is driven by the hydrolysis of ATP; when it's ATP bound it's bound/ fixed on the microtubules after hydrolysis it dissociates and alternates its position(same thing occurs with the other" leg"/ extension)

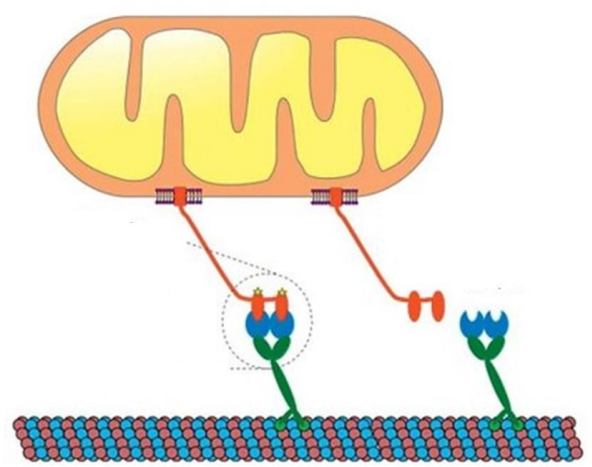
* the alteration between ATP hydrolysis and ATP binding drives the movement of the proteins*

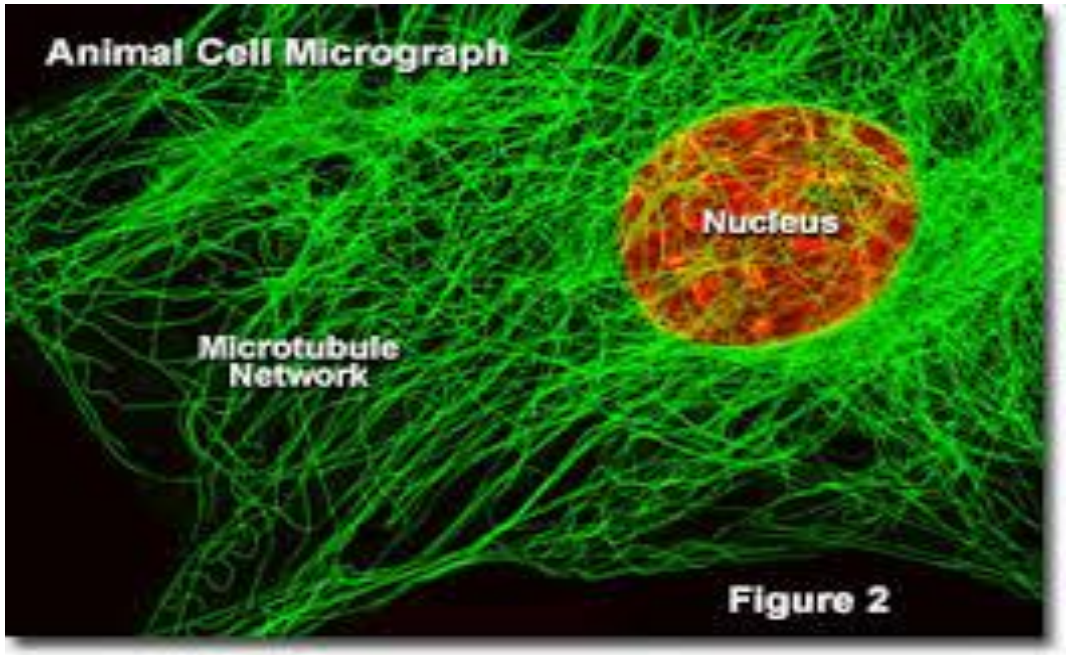


Other functions of microtubules

- Microtubules and their associated motor proteins position membrane-enclosed organelles(meaning they don't swim in the cytosol but rather bind to the microtubules and move throughout the cell(I highly recommend watching video 4 in references)) (such as the ER, Golgi apparatus, lysosomes, peroxisomes, and mitochondria) within the cell.

The mitochondria aren't found randomly they're focused at specific places.



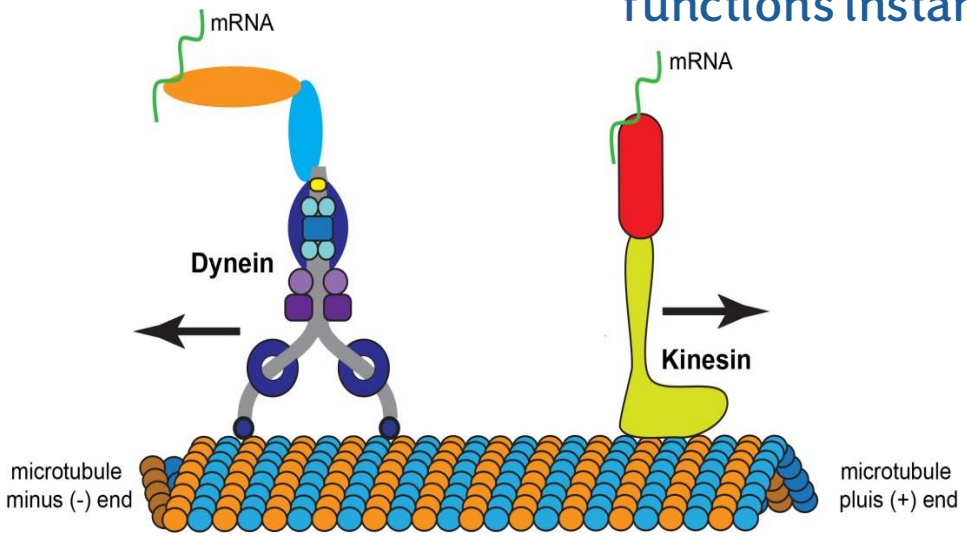


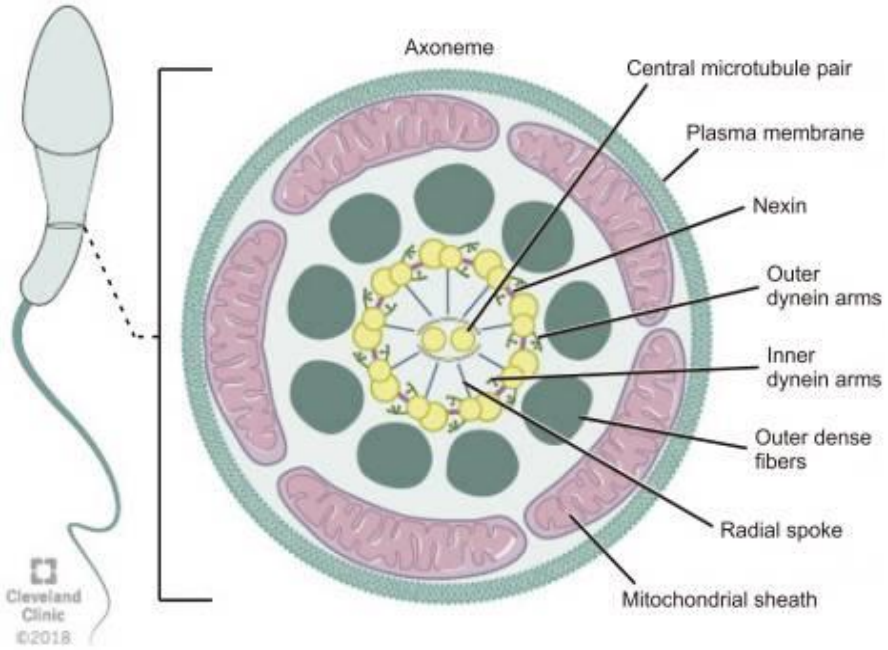
Look at how the microtubules are holding the nucleus in place(in its specific place) and of course, the nuclear-associated organelles like the ER and Golgi so they're not only tracks for vesicles and molecules but to hold and stabilize the cell's structure

Other functions of microtubules

أقرأ و استمتع: if you get it great if not skip <3
And note that the prof. Initially said" idk"
sb asked how microtubules determine when to polymerize and depolymerize(and all the other dynamic activity), well it's coordinated by MAPs and motor proteins.
And the coordination(**could be**) is driven by pulses of Ca++ that move the protein or simultaneous activation of ATP hydrolyzing enzymes=> second messenger

- Microtubules are responsible for sperm motility.
  - Infertility! Caused by mutations in the microtubules' motor protein(remember embryo :/)
- Kinesin and dynein transport selective mRNA molecules in cells. Suppose we must have a peripheral translation so the protein exits and functions instantly

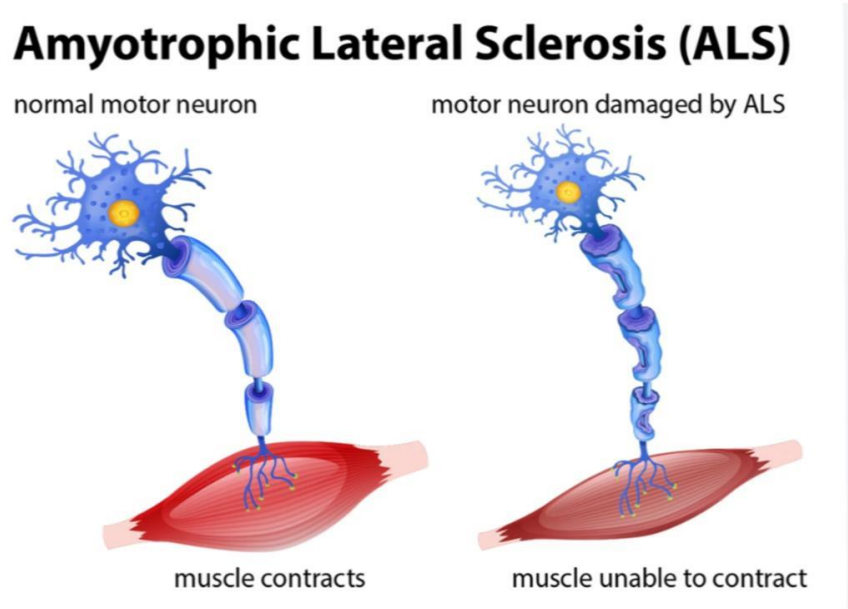




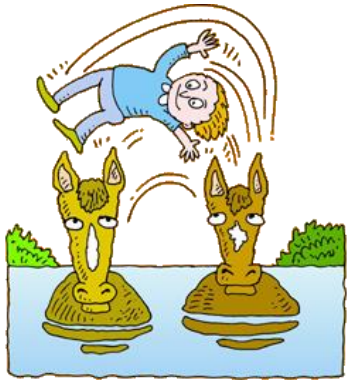
The sperm tail consists of microtubules and its constant movement is due to ATP hydrolysis-> dynamic instability; polymerization, and depolymerization, ... and this is similar mechanism for primitive organisms that have flagella

Kinesins and diseases

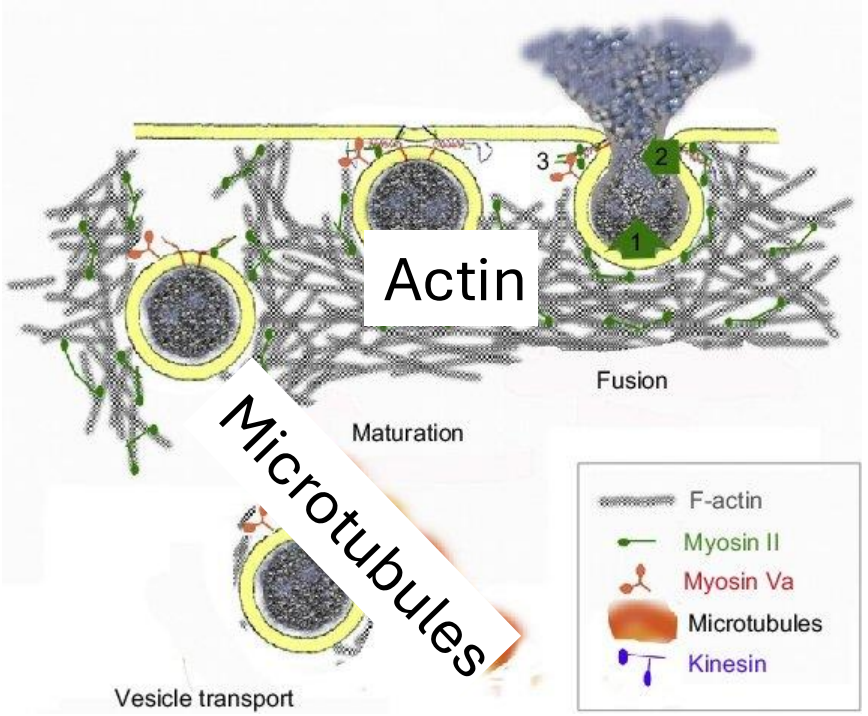
- Mutants in certain kinesin proteins reduce the ability of neurons to move essential organelles and proteins from their cell bodies to their axons there are two ways of synthesizing NT. Mechanism 1: to transport NT from the cell body to the synapses(the molecule binds to MT). Mechanism 2: to transport them via vesicles towards the synapses(vesicle binds to MT)) leading to neurodegeneration such as in amyotrophic lateral sclerosis (ALS; loss of muscle control) and Alzheimer's disease (dementia).
- Mutations in kinesins lead to peripheral neuropathies such as Charcot-Marie-Tooth disease(Lamin A mutations as well which is an intermediate filament=> cytoskeletal mutations).



“Changing horses in midstream”



- Myosins of actin filaments transport organelles over shorter distances compared to microtubules’s kinesins and dyneins.
- Kinesins and myosins transport organelles from the center of the cell towards the periphery, where myosins take over moving organelles near the plasma membrane.



Next slide ↓

“Changing horses in midstream”

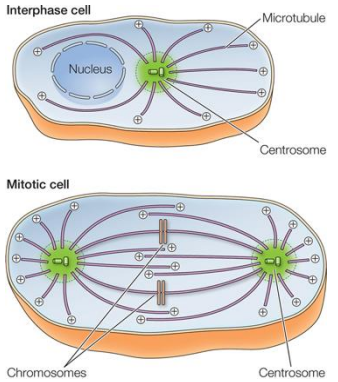
- There is a coordination between microtubules and actin cytoskeleton in transporting vesicles.
- Vesicles bind through **kinesins** via the microtubules and they are transported all the way to the end,, but microtubules don’t extend to the plasma membrane, but actin does
- So we have the actin cortex and the bundle that associate the cortex with plasma membrane
- So **1)** vesicle move along the microtubules and stops at the actin cytoskeleton, then **2)** the vesicle jumps from microtubules to the actin –> interacting with myosin
- “Myosin is a protein in muscles responsible for contraction and has many types”
- And then **3)** they get fused with the plasma membrane
- So there is a coordination between kinesins and myosin in **A)** transporting vesicles and **B)** the mechanism of fusion with plasma membrane

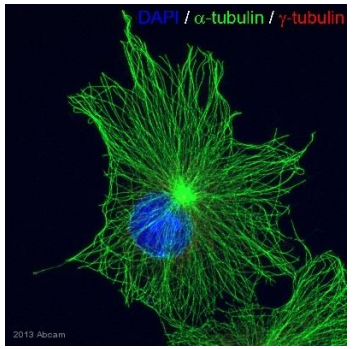
- The interaction between actin cytoskeleton and plasma membrane is regulated by **ca2+**
- In nerve cells the cell that carries neurotransmitters –> they are transported through microtubules and bind to the actin, but there is a gap between the actin and the plasma membrane preventing it to fuse until the influx of ca2+, ca2+ binds with actin binding proteins then actin is associated with plasma membrane.
- so we have made the bridge in which the vesicle can be fused with the plasma membrane and ca2+ do tethering by making the SNAREs closer to each other.

Centrosome

A microtubule-organizing center

- The centrosome serves as the initiation site for the assembly of microtubules, which then grow outward toward the periphery of the cell with their minus ends anchored in the centrosome.
  - In interphase cells, the centrosome is located near the nucleus and microtubules extend outward to the cell periphery.
  - During mitosis, duplicated centrosomes separate, and microtubules reorganize to form the mitotic spindle.





→ Is made of (GAMA-tubuline)

→ Initiaion site for microtubules assembly

→ It is a specialized organelle

1 centrosome division happens and each one is directed towards one end of the cell

2 Microtubules are extended from centrioles, The (+ end) of the microtubule will be in the center of the cell and the (- end) will be in the centriole itself

3 Microtubules interact with chromosomes, and then shrinkage (depolymerization) happens and it pulls the chromosomes to both ends of the cell.

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

1. https://youtu.be/XYZf-PYyGWA?si=kj3w_wna-2luoLiT
2. https://youtu.be/tO-W8mvBa78?si=09KA5p8cB1mdAIW2
3. https://youtu.be/fBGGWvj1oKU?si=l_IS1O_xwEGfg1kB
4. https://youtu.be/RRfH4ixgJwg?si=vsSeGZ6pZPv5Sbok

رسالة من الفريق العلمي:

- ارضَ بما قسمه الله لك تكن أغنى الناس.. ومن رضي فله الرضا
- لا تترك نفسك لسوء الأفكار.. وعارضها بتذكر ألطاف الله السابقة وصفات الله الثابتة
- اللهم فرّج كرب إخواننا في غزة ولبنان والسودان وآمنهم في بيوتهم واكفهم شرّ الأعداء